10/079,478.

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Doran R. Pace, Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. UF-178AXC2
Patent No. 6,809,235

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	L. Curtis Hannah, Thomas W. Greene
Issued	:	October 26, 2004
Patent No.	:	6,809,235 B2
For	:	Heat Stable Mutants of Starch Biosynthesis Enzymes

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 1, line 46:	Page 2, Line 3:
"25/150°C"	-- 25/15°C --.
Column 3, line 19:	Page 4, line 19:
"(1 12:1315-1320)"	-- (112:1315-1320) --.

M:\SherryL\doc\COC\UF-178AXC2.doc/DNB/amh

Column 3, line 59:

"(Bac, J.M.,"

Column 3, line 65:

"[1992] (1992) Plant"

Column 4, line 7:

"Sh2 and B:2"

Column 5, line 15:

"(SEQ ID NO:2')"

Column 5, line 24:

"Okita, T.W. Plant"

Column 5, line 27:

"Preiss, J. J. Biol."

Column 5, line 48:

"amino avid"

Column 14, line 30:

" use of Dpn1"

Column 16, line 19:

"*gig* A⁻ strain"

Column 16, line 25:

"*gig* A⁻ strain"

Page 5, line 17:

-- (Bae, J.M., --.

Page 5, line 21:

-- [1992] Plant --.

Page 5, line 27:

-- *Sh2* and *Bt2* --.

Amendment dated December 8, 2003, Page 3 (substituting page 7, line 22):

-- (SEQ ID NO:2) --.

Page 7, line 28:

-- Okita, T.W. [1996] Plant --.

Page 7, line 29:

-- Preiss, J. [1994] J. Biol. --.

Page 8, line 13:

-- amino acid --.

Page 23, line 6:

-- use of DpnI --.

Page 26, line 15:

-- *glg* A⁻ strain --.

Page 26, lines 18 and 19:

-- *glg* A⁻ strain --.

Column 31, line 15:	Amendment dated December 8, 2003 (original claim 47, renumbered as claim 7):
"one seine residue"	-- one serine residue --.
Column 32, line 23:	Amendment dated December 8, 2003 (original claim 69, renumbered as claim 24):
"herein"	-- wherein --.
Column 32, line 43:	Amendment dated May 24, 2004 (original claim 86, renumbered as claim 28):
"one seine residue"	-- one serine residue --.

A true and correct copy of pages 2, 4, 5, 7, 8, 23, and 26 of the specification, as filed, and Applicants' Amendment Under 37 CFR §1.111 dated December 8, 2003 and Amendment Under 37 CFR §1.116 dated May 24, 2004, which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

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DRP/amh
Attachments: Certificate of Correction; pages 2, 4, 5, 7, 8, 23, and 26 of the specification; Amendment Under 37 CFR §1.111 dated December 8, 2003; Amendment Under 37 CFR §1.116 dated May 24, 2004

Badu-Apraku *et al.* (Badu-Apraku, B., Hunter, R. B., and Tollenaar, M. [1983] *Can. J. Plant. Sci.* 63:357-363) measured a marked reduction in the yield of maize plants grown under the day/night temperature regime of 35/15° C compared to growth in a 25/15° C temperature regime. Reduced yields due to increased temperatures is also supported by historical as well as climatological studies (Thompson, L. M. [1986] *Agron. J.* 78:649-653; Thompson, L. M. [1975] *Science* 188:535-541; Chang, J. [1981] *Agricul. Metero.* 24:253-262; and Conroy, J. P., Seneweera, S., Basra, A. S., Rogers, G., and Nissen-Wooller, B. [1994] *Aust. J. Plant Physiol.* 21:741-758).

That the physiological processes of the developing seed are adversely affected by heat stress is evident from studies using an *in vitro* kernel culture system (Jones, R.J., Gengenbach, B.G., and Cardwell, V.B. [1981] *Crop Science* 21:761-766; Jones, R.J., Ouattar, S., and Crookston, R.K. [1984] *Crop Science* 24:133-137; and Cheikh, N., and Jones, R.J. [1995] *Physiol. Plant.* 95:59-66). Maize kernels cultured at the above-optimum temperature of 35° C exhibited a dramatic reduction in weight.

Work with wheat identified the loss of soluble starch synthase (SSS) activity as a hallmark of the wheat endosperm's response to heat stress (Hawker, J. S. and Jenner, C. F. [1993] *Aust. J. Plant Physiol.* 20:197-209; Denyer, K., Hylton, C. M., and Smith, A. M. [1994] *Aust. J. Plant Physiol.* 21:783-789; Jenner, C. F. [1994] *Aust. J. Plant Physiol.* 21:791-806). Additional studies with SSS of wheat endosperm show that it is heat labile (Rijven, A.H.G.C. [1986] *Plant Physiol.* 81:448-453; Keeling, P.L., Bacon, P.J., Holt, D.C. [1993] *Planta.* 191:342-348; Jenner, C. F., Denyer, K., and Guerin, J. [1995] *Aust. J. Plant Physiol.* 22:703-709).

The roles of SSS and ADP glucose pyrophosphorylase (AGP) under heat stress conditions in maize is less clear. (AGP) catalyzes the conversion of ATP and α-glucose-1-phosphate to ADP-glucose and pyrophosphate. ADP-glucose is used as a glycosyl donor in starch biosynthesis by plants and in glycogen biosynthesis by bacteria. The importance of ADP-glucose pyrophosphorylase as a key enzyme in the regulation of starch biosynthesis was noted in the study of starch deficient mutants of maize (*Zea mays*) endosperm (Tsai, C.Y.,

Biochemical and genetic evidence has identified AGP as a key enzyme in starch biosynthesis in higher plants and glycogen biosynthesis in *E. coli* (Preiss, J. and Romeo, T. [1994] *Progress in Nuc. Acid Res. and Mol Biol.* 47:299-329; Preiss, J. and Sivak, M. [1996] "Starch synthesis in sinks and sources," In *Photoassimilate distribution in plants and crops: source-sink relationships*. Zamski, E., ed., Marcil Dekker Inc. pp. 139-168). AGP catalyzes what is viewed as the initial step in the starch biosynthetic pathway with the product of the reaction being the activated glucosyl donor, ADPglucose. This is utilized by starch synthase for extension of the polysaccharide polymer (reviewed in Hannah, L. Curtis [1996] "Starch synthesis in the maize endosperm," In: *Advances in Cellular and Molecular Biology of Plants*, Vol. 4. B. A. Larkins and I. K. Vasil (eds.). Cellular and Molecular Biology of Plant Seed Development. Kluwer Academic Publishers, Dordrecht, The Netherlands).

Initial studies with potato AGP showed that expression in *E. coli* yielded an enzyme with allosteric and kinetic properties very similar to the native tuber enzyme (Iglesias, A., Barry, G.F., Meyer, C., Bloksberg, L., Nakata, P., Greene, T., Laughlin, M.J., Okita, T.W., Kishore, G.M., and Preiss, J. [1993] *J. Biol Chem.* 268:1081-86; Ballicora, M.A., Laughlin, M.J., Fu, Y., Okita, T.W., Barry, G.F., and Preiss, J. [1995] *Plant Physiol.* 109:245-251). Greene *et al.* (Greene, T.W., Chantler, S.E., Kahn, M.L., Barry, G.F., Preiss, J., and Okita, T.W. [1996] *Proc. Natl. Acad. Sci.* 93:1509-1513; Greene, T.W., Woodbury, R.L., and Okita, T.W. [1996] *Plant Physiol.* (112:1315-1320) showed the usefulness of the bacterial expression system in their structure-function studies with the potato AGP. Multiple mutations important in mapping allosteric and substrate binding sites were identified (Okita, T.W., Greene, T.W., Laughlin, M.J., Salamone, P., Woodbury, R., Choi, S., Ito, H., Kavakli, H., and Stephens, K. [1996] "Engineering Plant Starches by the Generation of Modified Plant Biosynthetic Enzymes," In *Engineering Crops for Industrial End Uses*, Shewry, P.R., Napier, J.A., and Davis, P., eds., Portland Press Ltd., London).

AGP enzymes have been isolated from both bacteria and plants. Bacterial AGP consists of a homotetramer, while plant AGP from photosynthetic and non-photosynthetic tissues is a heterotetramer composed of two different subunits. The plant enzyme is encoded

by two different genes, with one subunit being larger than the other. This feature has been noted in a number of plants. The AGP subunits in spinach leaf have molecular weights of 54 kDa and 51 kDa, as estimated by SDS-PAGE. Both subunits are immunoreactive with antibody raised against purified AGP from spinach leaves (Copeland, L., J. Preiss (1981) *Plant Physiol.* 68:996-1001; Morell, M., M. Bloon, V. Knowles, J. Preiss [1988] *J. Bio. Chem.* 263:633). Immunological analysis using antiserum prepared against the small and large subunits of spinach leaf showed that potato tuber AGP is also encoded by two genes (Okita *et al.*, 1990, *supra*). The cDNA clones of the two subunits of potato tuber (50 and 51 kDa) have also been isolated and sequenced (Muller-Rober, B.T., J. Kossmann, L.C. Hannah, L. Willmitzer, U. Sounewald [1990] *Mol. Gen. Genet.* 224:136-146; Nakata, P.A., T.W. Greene, J.M. Anderson, B.J. Smith-White, T.W. Okita, J. Preiss [1991] *Plant Mol. Biol.* 17:1089-1093). The large subunit of potato tuber AGP is heat stable (Nakata *et al.* [1991], *supra*).

As Hannah and Nelson (Hannah, L.C., O.E. Nelson (1975) *Plant Physiol.* 55:297-302.; Hannah, L.C., and Nelson, Jr., O.E. [1976] *Biochem. Genet.* 14:547-560) postulated, both *Shrunken-2* (*Sh2*) (Bhave, M.R., S. Lawrence, C. Barton, L.C. Hannah [1990] *Plant Cell* 2:581-588) and *Brittle-2* (*Bt2*) (Bae, J.M., M. Giroux, L.C. Hannah [1990] *Maydica* 35:317-322) are structural genes of maize endosperm ADP-glucose pyrophosphorylase. *Sh2* and *Bt2* encode the large subunit and small subunit of the enzyme, respectively. From cDNA sequencing, *Sh2* and *Bt2* proteins have predicted molecular weight of 57,179 Da (Shaw, J.R., L.C. Hannah [1992] *Plant Physiol.* 98:1214-1216) and 52,224 Da, respectively. The endosperm is the site of most starch deposition during kernel development in maize. *Sh2* and *bt2* maize endosperm mutants have greatly reduced starch levels corresponding to deficient levels of AGP activity. Mutations of either gene have been shown to reduce AGP activity by about 95% (Tsai and Nelson, 1966, *supra*; Dickinson and Preiss, 1969, *supra*). Furthermore, it has been observed that enzymatic activities increase with the dosage of functional wild type *Sh2* and *Bt2* alleles, whereas mutant enzymes have altered kinetic properties. AGP is the rate limiting step in starch biosynthesis in plants. Stark *et al.* placed a mutant form of *E. coli* AGP

in a functionally important region of maize endosperm AGP. One mutant, *Rev 6,* contained a tyrosine-serine insert in the large subunit of AGP and conditioned a 11-18% increase in seed weight. In addition, published international application WO 01/64928 teaches that various characteristics, such as seed number, plant biomass, Harvest Index *etc.*, can be increased in plants transformed with a polynucleotide encoding a large subunit of maize AGP containing the *Rev6* mutation.

Brief Summary of the Invention

The subject invention pertains to materials and methods useful for improving crop yields in plants, such as those plants that produce cereal crops. In one embodiment, the subject invention provides heat stable AGP enzymes and nucleotide sequences which encode these enzymes. In a preferred embodiment, the heat stable enzymes of the invention can be used to provide plants having greater tolerance to higher temperatures, thus enhancing the crop yields from these plants. In a particularly preferred embodiment, the improved plant is a cereal. Cereals to which this invention applies include, for example, maize, wheat, rice, and barley.

Brief Description of the Drawings

Figure 1 shows heat stable maize endosperm AGP large subunit mutants. Percentage of AGP activity remaining after five minutes of heat treatment at 60° C is shown.

Figure 2 shows primary sequence alignment of the region surrounding HS 33 mutation in the AGP large subunits for maize, wheat, barley, and potato. Conserved regions are boxed.

Figure 3 shows primary sequence alignment of the region surrounding HS 40 mutation in the AGP large subunits for maize, wheat, barley, and potato. Conserved regions are boxed. Bolded aspartic acid residue corresponds to D413A allosteric mutant of potato LS (Greene, T.W., Woodbury, R.L., and Okita, T.W. [1996] *Plant Physiol.* (112:1315-1320). Spinach leaf AGP sequence is the activator site 2 peptide identified in 3-PGA

analogue studies (Ball, K. and Preiss, J. [1994] *J. Biol. Chem.* 269:24706-24711). The labeled lysine residue is bolded.

Figures 4A and 4B show molecular characterization of TS48 and TS60, respectively. Genetic lesion of TS48 and corresponding residues are in bold. The amino acid number is indicated above the Leu to Phe mutation of TS48. The last line is a consensus sequence. The Leu residue is highly conserved. Genetic lesions of TS60 and corresponding residues are in bold. The amino acid numbers are indicated above the Glu to Lys and Ala to Val mutations of TS60. Boxed residues correspond to the HS 33 mutation previously identified and shown to be important in heat stability of the maize endosperm AGP. The last line is a consensus sequence.

Figures 5A and 5B show molecular characterization of RTS 48-2 and RTS 60-1, respectively. Genetic lesion of RTS 48-2 and corresponding residues are in bold. The amino acid number is indicated above the Ala to Val mutation of RTS 48-2. The last line is a consensus sequence. Of significance, the mutation identified in RTS 48-2 maps to the identical residue found in the heat stable variant HS13. HS 13 contained an Ala to Pro mutation at position 177. Genetic lesion of RTS 60-1 and corresponding residues are in bold. The amino acid number is indicated above the Ala to Val mutation of RTS 60-1. The last line is a consensus sequence.

Brief Description of the Sequences

SEQ ID NO. 1 is an amino acid sequence of a region of the large subunit of AGP in maize containing the HS 33 mutation as shown in Figure 2.

SEQ ID NO. 2 is an amino acid sequence of a region of the large subunit of AGP in maize as shown in Figure 2.

SEQ ID NO. 3 is an amino acid sequence of a region of the large subunit of AGP in wheat as shown in Figure 2.

SEQ ID NO. 4 is an amino acid sequence of a region of the large subunit of AGP in barley as shown in Figure 2.

Multiple HS mutants within one subunit can easily be combined. For example, different unique restriction sites that divide the coding regions of *Sh2* into three distinct fragments can be used. Where appropriate, mutation combinations can be generated by subcloning the corresponding fragment containing the added mutation. If two mutations are in close proximity, then site-directed mutagenesis can be used to engineer such combinations. One method for site specific mutations involves PCR, mutagenic primer, and the use of *Dpn*I restriction endonuclease. Primers can be constructed to contain the mutation in the 5′ end, and used to PCR amplify using the proofreading polymerase Vent. Amplified DNA can then be digested with *Dpn*I. Parental DNA isolated from *E. coli* is methylated and hence susceptible to *Dpn*I. Digested DNA is size fractionated by gel electrophoresis, ligated, and cloned into the expression vectors. Mutations are confirmed by sequence analysis and transformed into the AC70R1-504 strain carrying the wild-type small subunit. Combinatorial mutants can then be analyzed.

Example 6 – Identification of Additional Mutants at Position 333 of the Large Subunit of Maize AGP

Hydroxylamine-HCl mutagenesis gives rise only to cytosine to thymine changes, thereby limiting the types of possible substitutions. Because both strands of DNA undergo mutagenesis, thymine to cytosine changes also occur; however, taken together, only two of the 12 possible single base changes occur. Hence, not all possible amino acid substitutions would have been produced by hydroxylamine-HCl mutagenesis.

Therefore, in order to prepare mutants where each of the 20 different amino acids were inserted, individually, at position 333 of the large subunit of maize endosperm AGP, a two step process was employed. Methodologies were derived basically from those of Stratagene. First, the codon encoding amino acid 333, plus the first base of the codon for amino acid 334, were removed via PCR-based site-specific mutagenesis (Suzuki *et al.*, 1989, *supra*). Following screening for inactivity by iodine staining and subsequent sequencing to verify the deletion, the resulting plasmid was PCR mutagenized using a primer containing

Example 8 – Cloning of SSS I Mutants

A *glg* A⁻ *E. coli* strain deficient in the endogenous bacterial glycogen synthase can be obtained from the *E. coli* Stock Center. Bacterial expression vectors currently used for the expression of AGP can be used for expression of SSS.

One cloning strategy, as used, for example, with *Sh2* and *Bt2* (Giroux *et al.*, 1996, *supra*), is the following: One primer contains a unique restriction plus the 5′ terminus of the transcript while the other primer contains another unique restriction site and sequences 3′ to the translational termination codon of the gene under investigation. Subsequent cloning of these gives rise to a translational fusion within the plasmid. These gene specific primers are initially used in RT-PCR reactions using poly A+RNA from developing endosperms.

Expression of the maize endosperm SSS I will complement the lack of glycogen synthase activity in the *glg* A⁻ strain. Complementation should be easily visualized with iodine staining as it is with the expression of AGP in the *glg* C⁻ strain. Crude extracts can be incubated at various temperatures and lengths of time to determine the heat stability of SSS I. The *glg* A⁻ strain expressing the maize endosperm SSS I can be grown at various temperatures to determine if function is temperature sensitive as it is with the AGP bacterial expression system. Once a restrictive temperature is established, a random mutagenesis can be conducted with the SSS I clone. Mutant forms of SSS I can be transformed into the *glg* A⁻ strain, grown at the restrictive temperature, and heat stable variants identified by their ability to produce iodine-staining glycogen at the restriction temperature.

Example 9 – Temperature sensitive mutants of maize endosperm ADP-glucose pyrophosphorylase

As an alternative approach to identify additional variants with increased stability, a reverse-genetics approach was employed. Temperature sensitive (TS) mutants have been isolated. These mutants exhibit a negative iodine staining phenotype at 30° C indicating a lack of function with the maize endosperm AGP. In contrast, when the mutants are grown at 37° C they can fully complement the mutation in the bacterial AGP. This clearly shows

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Doran R. Pace, Patent Attorney

AMENDMENT UNDER 37 CFR §1.116
Examining Group 1638
Patent Application
Docket No. UF-178AXC2
Serial No. 10/079,478

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Anne R. Kubelik, Ph.D.
Art Unit	:	1638
Applicants	:	L. Curtis Hannah, Thomas W. Greene
Serial No.	:	10/079,478
Filed	:	February 19, 2002
Conf. No.	:	7981
For	:	Heat Stable Mutants of Starch Biosynthesis Enzymes

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

AMENDMENT UNDER 37 CFR §1.116

Sir:

In response to the Office Action dated February 24, 2004, please amend the above-identified patent application as follows:

Claims

1. (currently amended) A purified polynucleotide comprising a nucleotide sequence encoding a full-length mutant polypeptide of ~~large subunit of a plant~~ maize ADP-glucose pyrophosphorylase ~~polypeptide~~ large subunit, or a ~~biologically active~~ fragment of said full-length mutant polypeptide, wherein said full-length mutant polypeptide or said fragment of said full-length mutant polypeptide comprises an amino acid mutation in the amino acid sequence of said ~~polypeptide~~ maize ADP-glucose pyrophosphorylase large subunit, and wherein when said full-length mutant polypeptide or said fragment of said full-length mutant polypeptide is expressed to form a mutant ADP-glucose pyrophosphorylase enzyme, said mutant enzyme~~, or a fragment of said mutant enzyme~~, exhibits increased heat stability relative to the wild type ADP-glucose pyrophosphorylase enzyme.

2-41. (canceled)

42. (currently amended) The polynucleotide according to claim 1, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the ~~amino acid corresponding to~~ histidine at position 333 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

43. (currently amended) The polynucleotide according to claim 42, wherein said ~~amino acid corresponding to~~ histidine at position 333 is replaced with a phenylalanine.

44. (currently amended) The polynucleotide according to claim 42, wherein said ~~amino acid corresponding to~~ histidine at position 333 is replaced with a methionine.

45. (previously presented) The polynucleotide according to claim 1, wherein said mutant polypeptide encoded by said polynucleotide further comprises an amino acid mutation that confers increased seed weight to a plant expressing said polynucleotide.

46. (previously presented) The polynucleotide according to claim 45, wherein said polynucleotide comprises the *Rev6* mutation.

47. (currently amended) The polynucleotide according to claim 45, wherein said mutation comprises the insertion of at least one serine residue between ~~amino acids corresponding to positions~~ the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

48. (currently amended) The polynucleotide according to claim 45, wherein said mutation comprises the insertion of the amino acid pair tyrosine:serine between ~~amino acids corresponding to positions~~ the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

49. (currently amended) The polynucleotide according to claim 45, wherein said mutation comprises the insertion of the amino acid pair serine:tyrosine between ~~amino acids corresponding to positions~~ the tyrosine at position 495 and the tyrosine at position 496 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

50-54. (canceled)

55. (currently amended) The polynucleotide according to claim 1, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the ~~amino acid corresponding to~~ leucine at position 426 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a phenylalanine amino acid and the ~~amino acid corresponding to~~ alanine at position 177 of the wild type large subunit of ADP-glucose

pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

56. (currently amended) The polynucleotide according to claim 55, wherein said ~~amino acid corresponding to~~ alanine at position 177 is replaced with a proline.

57. (currently amended) The polynucleotide according to claim 55, wherein said ~~amino acid corresponding to~~ alanine at position 177 is replaced with a valine.

58. (currently amended) The polynucleotide according to claim 1, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the ~~amino acid corresponding to~~ glutamic acid at position 324 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a lysine amino acid, and the ~~amino acid corresponding to~~ alanine at position 359 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a valine amino acid, and the ~~amino acid corresponding to~~ alanine at position 396 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

59. (currently amended) The polynucleotide according to claim 58, wherein said ~~amino acid corresponding to~~ alanine at position 396 is replaced with a valine.

60. (previously presented) A method for increasing resistance of a plant to heat stress conditions, said method comprising incorporating the polynucleotide of claim 1 into the genome of said plant and expressing the protein encoded by said polynucleotide molecule.

61. (previously presented) The method according to claim 60, wherein said plant is a monocotyledonous plant.

62. (previously presented) The method according to claim 61, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, lily, and millet.

63. (previously presented) The method according to claim 60, wherein said plant is *Zea mays*.

64. (previously presented) The method according to claim 60, wherein said plant is a dicotyledonous plant.

65. (previously presented) The method according to claim 64, wherein said dicotyledonous plant is selected from the group consisting of pea, alfalfa, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple tree, and lettuce.

66. (currently amended) The method according to claim 60, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the ~~amino acid corresponding to~~ leucine at position 426 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a phenylalanine amino acid and the ~~amino acid corresponding to~~ alanine at position 177 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

67. (currently amended) The method according to claim 66, wherein said ~~amino acid corresponding to~~ alanine at position 177 is replaced with a proline.

68. (currently amended) The method according to claim 66, wherein said ~~acid corresponding to~~ alanine at position 177 is replaced with a valine.

69. (currently amended) The method according to claim 60, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the ~~amino acid corresponding to~~ glutamic acid at position 324 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a lysine amino acid, and the ~~amino acid corresponding to~~ alanine at position 359 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a valine amino acid, and the ~~amino acid corresponding to~~ alanine at position 396 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

70. (currently amended) The method according to claim 69, wherein said ~~amino acid corresponding to~~ alanine at position 396 is replaced with a valine.

71. (previously presented) The method according to claim 60, wherein said mutant polypeptide encoded by said polynucleotide further comprises an amino acid mutation that confers increased seed weight to a plant expressing said polynucleotide.

72. (previously presented) A plant or plant tissue comprising the polynucleotide molecule of claim 1.

73. (previously presented) The plant or plant tissue according to claim 72, wherein said plant or plant tissue is monocotyledonous.

74. (previously presented) The plant or plant tissue according to claim 73, wherein said monocotyledonous plant or plant tissue is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, lily, and millet.

75. (previously presented) The plant or plant tissue according to claim 72, wherein said plant is *Zea mays* or said plant tissue is from *Zea mays*.

76. (previously presented) The plant or plant tissue according to claim 72, wherein said plant or plant tissue is dicotyledonous.

77. (previously presented) The plant or plant tissue according to claim 76, wherein said dicotyledonous plant or plant tissue is selected from the group consisting of pea, alfalfa, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple tree, and lettuce.

78. (previously presented) The plant tissue according to claim 72, wherein said plant tissue is a seed.

79. (currently amended) The plant or plant tissue according to claim 72, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the ~~amino acid corresponding to~~ leucine at position 426 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a phenylalanine amino acid and the ~~amino acid corresponding to~~ alanine at position 177 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

80. (currently amended) The plant or plant tissue according to claim 79, wherein said ~~amino acid corresponding to~~ alanine at position 177 is replaced with a proline.

81. (currently amended) The plant or plant tissue according to claim 79, wherein said ~~amino acid corresponding to~~ alanine at position 177 is replaced with a valine.

82. (currently amended) The plant or plant tissue according to claim 72, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the ~~amino acid corresponding to~~ glutamic acid at position 324 of the wild type large subunit of ADP-glucose

pyrophosphorylase polypeptide of maize is replaced by a lysine amino acid, and the ~~amino acid corresponding to~~ alanine at position 359 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a valine amino acid, and the ~~amino acid corresponding to~~ alanine at position 396 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

83. (currently amended) The plant or plant tissue according to claim 82, wherein said ~~amino acid corresponding to~~ alanine at position 396 is replaced with a valine.

84. (previously presented) The plant or plant tissue according to claim 72, wherein said mutant polypeptide encoded by said polynucleotide further comprises an amino acid mutation that confers increased seed weight to a plant expressing said polynucleotide.

85. (new) The method according to claim 71, wherein said polynucleotide comprises the *Rev6* mutation.

86. (new) The method according to claim 71, wherein said mutation comprises the insertion of at least one serine residue between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

87. (new) The method according to claim 71, wherein said mutation comprises the insertion of the amino acid pair tyrosine:serine between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

88. (new) The method according to claim 71, wherein said mutation comprises the insertion of the amino acid pair serine:tyrosine between the tyrosine at position 495 and the tyrosine at

position 496 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

89. (new) The plant or plant tissue according to claim 84, wherein said polynucleotide comprises the *Rev6* mutation.

90. (new) The plant or plant tissue according to claim 84, wherein said mutation comprises the insertion of at least one serine residue between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

91. (new) The plant or plant tissue according to claim 84, wherein said mutation comprises the insertion of the amino acid pair tyrosine:serine between the glycine at position 494 and the tyrosine at position 495 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

92. (new) The plant or plant tissue according to claim 84, wherein said mutation comprises the insertion of the amino acid pair serine:tyrosine between the tyrosine at position 495 and the tyrosine at position 496 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

Remarks

Claims 1 and 42-84 are pending in the subject application. As an initial matter, Applicants gratefully acknowledge the Examiner's withdrawal of the rejections under 35 USC §101 and 35 USC §102(b). By this Amendment, Applicants have amended claims 1, 42-44, 47-49, 55-59, 66-70, and 79-83, canceled claims 50-54, and added new claims 85-92. Support for the new claims and amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the new claims and amendments presented herein is respectfully requested. Accordingly, claims 1, 42-49, and 55-92 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

Claims 1, 42-51, and 53-84 are rejected under 35 USC §112, first paragraph, as nonenabled by the subject specification and as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner acknowledges that the specification is enabled and provides written description for polynucleotides that encode mutant ADP-glucose pyrophosphorylase (AGP) large subunits from maize. However, the Examiner asserts that the subject specification does not enable and does not provide written description for polynucleotides encoding heat stable mutants of AGP large subunit from plants other than maize.

Applicants respectfully assert that the claims are enabled by the subject specification and that there is adequate written description in the subject specification to convey to the ordinarily skilled artisan that they had possession of the claimed invention. However, in order to expedite prosecution of the subject invention, Applicants have amended claim 1 so that the claim now recites a polynucleotide encoding a mutant maize ADP-glucose pyrophosphorylase large subunit. As noted above, the Examiner has acknowledged that there is enablement and written description for polynucleotides encoding the large subunit of maize AGP. The method claims and the plant and plant tissue composition claims, by reference to the polynucleotide of claim 1, effectively recite that the polynucleotide encodes a mutant polypeptide of maize AGP. In addition, Applicants have canceled claims 50-54, which are directed to certain polynucleotides that encode heat stable AGP large subunit from various other plants. Applicants respectfully assert that an ordinarily skilled

artisan, having the benefit of the teachings of the subject application, can readily practice the claimed invention without resort to undue experimentation. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §112, first paragraph, is respectfully requested.

Claims 1 and 42-84 are rejected under 35 USC §112, second paragraph, as indefinite. Applicants respectfully submit that the claims are definite. In regard to the Examiner's assertion that the recitation of "biologically active fragment" renders claim 1 indefinite, Applicants have amended the claim to delete reference to "biologically-active." The claim encompasses those fragments of the full-length mutant polypeptide, that when expressed to form a mutant AGP enzyme, the mutant enzyme exhibits increased heat stability relative to wild type enzyme. An ordinarily skilled artisan can readily prepare polynucleotides encoding fragments of the mutant large subunit of AGP and express the encoded fragment in a system to form the AGP enzyme and determine if the mutant enzyme exhibits increased heat stability. Also under this rejection, the Examiner asserts that the recitation of "amino acid corresponding to position(s)" renders claims 42-44, 47-49, 55-59, 66-70, and 79-83 indefinite. The Examiner asserts that it is unclear what it means for an amino acid to correspond to a position. By this Amendment, Applicants have amended claims 42-44, 47-49, 55-59, 66-70, and 79-83 to indicate a specific amino acid located at a specific position in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase is replaced by another amino acid. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §112, second paragraph, is respectfully requested.

Claims 1, 42, and 50-52 are rejected under the judicially created doctrine of "obviousness-type" double patenting over claim 1 of U.S. Patent No. 6,069,300. In addition, claims 1, 42, 50-52, 58-63, 69, 70, 72-75, 78, 82, and 83 are rejected under the judicially created doctrine of "obviousness-type" double patenting over claims 1-3, 20-30, and 48-54 of U.S. Patent No. 6,403,863. Applicants respectfully submit that the claims in the subject application are not obvious over the cited patents. However, in order to expedite prosecution of the subject application, Applicants have submitted a Terminal Disclaimer with this Amendment that obviates these rejections. Accordingly, reconsideration and withdrawal of the "obviousness-type" double patenting rejections is respectfully requested.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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DRP/sl
Attachment: Terminal Disclaimer

COPY

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on December 8, 2003.

Doran R. Pace, Patent Attorney

AMENDMENT UNDER 37 CFR §1.111
Examining Group 1638
Patent Application
Docket No. UF-178AXC2
Serial No. 10/079,478

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Anne R. Kubelik
Art Unit	:	1638
Applicants	:	L. Curtis Hannah, Thomas W. Greene
Serial No.	:	10/079,478
Filed	:	February 19, 2002
Conf. No.	:	7981
For	:	Heat Stable Mutants of Starch Biosynthesis Enzymes

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

AMENDMENT UNDER 37 CFR §1.111

Sir:

A Petition and Fee for a one-month Extension of Time through and including December 8, 2003, accompanies this Amendment.

In response to the Office Action dated August 8, 2003, please amend the above-identified patent application as follows:

In the Specification

Please substitute the following paragraph on page 1, beginning at line 9:

Cross-Reference to ~~a~~ Related Applications

This application is a continuation-in-part of ~~co-pending~~ U.S. Application No. 09/312,433, filed May 14, 1999, now U.S. Patent No. 6,403,863, which is a continuation-in-part of ~~co-pending~~ U.S. Application No. 08/972,545, filed November 18, 1997, now U.S. Patent No. 6,069,300. ~~This application also~~ U.S. Application No. 09/312,433 claims ~~priority from~~ the benefit of U.S. Provisional Application No. ~~60,085,460~~ 60/085,460, filed May 14, 1998 and U.S. Application No. 08/972,545 claims the benefit of U.S. Provisional Application No. 60/031,045, filed November 18, 1996.

Please substitute the following paragraph on page 5, beginning at line14 through to page 6, line 2:

As Hannah and Nelson (Hannah, L.C., O.E. Nelson (1975) *Plant Physiol.* ~~55:297-302.;~~ 55:297-302; Hannah, L.C., and Nelson, Jr., O.E. [1976] *Biochem. Genet.* 14:547-560) postulated, both *Shrunken-2* (*Sh2*) (Bhave, M.R., S. Lawrence, C. Barton, L.C. Hannah [1990] *Plant Cell* 2:581-588) and *Brittle-2* (*Bt2*) (Bae, J.M., M. Giroux, L.C. Hannah [1990] *Maydica* 35:317-322) are structural genes of maize endosperm ADP-glucose pyrophosphorylase. *Sh2* and *Bt2* encode the large subunit and small subunit of the enzyme, respectively. From cDNA sequencing, *Sh2* and *Bt2* proteins have predicted molecular weight of 57,179 Da (Shaw, J.R., L.C. Hannah [1992] *Plant Physiol.* 98:1214-1216) and 52,224 Da, respectively. The endosperm is the site of most starch deposition during kernel development in maize. *Sh2* and ~~*bt2*~~ *Bt2* maize endosperm mutants have greatly reduced starch levels corresponding to deficient levels of AGP activity. Mutations of either gene have been shown to reduce AGP activity by about 95% (Tsai and Nelson, 1966, *supra*; Dickinson and Preiss, 1969, *supra*). Furthermore, it has been observed that enzymatic activities increase with the dosage of functional wild type *Sh2* and *Bt2* alleles, whereas mutant enzymes have altered kinetic properties. AGP is the rate limiting step in starch biosynthesis in plants. Stark *et al.* placed a mutant form of *E. coli* AGP in potato tuber and obtained a 35% increase in starch content (Stark *et al.* [1992] *Science* 258:287).

Please substitute the following paragraphs on page 7, beginning on line 21 through to page 8, line 18:

Figure 2 shows primary sequence alignment of the region surrounding HS 33 mutation (SEQ ID NO:1) in the AGP large subunits for maize (SEQ ID NO:2), wheat (SEQ ID NO:3), barley (SEQ ID NO:4), and potato (SEQ ID NO:5). Conserved regions are boxed.

Figure 3 shows primary sequence alignment of the region surrounding HS 40 mutation (SEQ ID NO:6) in the AGP large subunits for maize (SEQ ID NO:7), wheat (SEQ ID NO:8), barley (SEQ ID NO:9), and potato (SEQ ID NO:10). Conserved regions are boxed. Bolded aspartic acid residue corresponds to D413A allosteric mutant of potato LS (Greene, T.W., Woodbury, R.L., and Okita, T.W. [1996] *Plant Physiol.* (112:1315-1320). Spinach leaf AGP sequence (SEQ ID NO:11) is the activator site 2 peptide identified in 3-PGA analogue studies (Ball, K. and Preiss, J. [1994] *J. Biol. Chem.* 269:24706-24711). The labeled lysine residue is bolded.

Figures 4A and 4B show molecular characterization of TS48 (SEQ ID NO:12) and TS60 (SEQ ID NO:17), respectively. Genetic lesion of TS48 (SEQ ID NO:12) and corresponding residues are in bold. The amino acid number is indicated above the Leu to Phe mutation of TS48 (SEQ ID NO:12). The last line is a consensus sequence. The Leu residue is highly conserved. Genetic lesions of TS60 (SEQ ID NO:17) and corresponding residues are in bold. The amino acid numbers are indicated above the Glu to Lys and Ala to Val mutations of TS60 (SEQ ID NO:17). Boxed residues correspond to the HS 33 mutation (SEQ ID NO:1) previously identified and shown to be important in heat stability of the maize endosperm AGP. The last line is a consensus sequence.

Figures 5A and 5B show molecular characterization of RTS 48-2 (SEQ ID NO:27) and RTS 60-1 (SEQ ID NO:32), respectively. Genetic lesion of RTS 48-2 (SEQ ID NO:27) and corresponding residues are in bold. The amino acid number is indicated above the Ala to Val mutation of RTS 48-2 (SEQ ID NO:27). The last line is a consensus sequence. Of significance, the mutation identified in RTS 48-2 (SEQ ID NO:27) maps to the identical residue found in the heat stable variant HS13. HS 13 contained an Ala to Pro mutation at position 177. Genetic lesion of RTS 60-1 (SEQ ID NO:32) and corresponding residues are in bold. The amino acid number is indicated above the Ala to Val mutation of RTS 60-1 (SEQ ID NO:32). The last line is a consensus sequence.

In the Claims

1 (currently amended). A purified polynucleotide comprising a nucleotide sequence encoding a mutant large subunit of a plant ADP-glucose pyrophosphorylase polypeptide, or a biologically-active fragment of said mutant polypeptide, wherein said mutant polypeptide comprises an amino acid mutation in the amino acid sequence of said polypeptide and wherein when said mutant polypeptide is expressed to form a mutant ADP-glucose pyrophosphorylase enzyme, said mutant enzyme, or a biologically-active fragment of said mutant enzyme, exhibits increased heat stability relative to the wild type ADP-glucose pyrophosphorylase enzyme.

Claims 2-41 (canceled)

42 (new). The polynucleotide according to claim 1, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the amino acid corresponding to position 333 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

43 (new). The polynucleotide according to claim 42, wherein said amino acid corresponding to position 333 is replaced with a phenylalanine.

44 (new). The polynucleotide according to claim 42, wherein said amino acid corresponding to position 333 is replaced with a methionine.

45 (new). The polynucleotide according to claim 1, wherein said mutant polypeptide encoded by said polynucleotide further comprises an amino acid mutation that confers increased seed weight to a plant expressing said polynucleotide.

46 (new). The polynucleotide according to claim 45, wherein said polynucleotide comprises the *Rev6* mutation.

47 (new). The polynucleotide according to claim 45, wherein said mutation comprises the insertion of at least one serine residue between amino acids corresponding to positions 494 and 495 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

48 (new). The polynucleotide according to claim 45, wherein said mutation comprises the insertion of the amino acid pair tyrosine:serine between amino acids corresponding to positions 494 and 495 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

49 (new). The polynucleotide according to claim 45, wherein said mutation comprises the insertion of the amino acid pair serine:tyrosine between amino acids corresponding to positions 495 and 496 in the amino acid sequence of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize.

50 (new). The polynucleotide according to claim 1, wherein said plant is a monocotyledonous plant.

51 (new). The polynucleotide according to claim 50, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, lily, and millet.

52 (new). The polynucleotide according to claim 1, wherein said plant is *Zea mays*.

53 (new). The polynucleotide according to claim 1, wherein said plant is a dicotyledonous plant.

54 (new). The polynucleotide according to claim 53, wherein said dicotyledonous plant is selected from the group consisting of pea, alfalfa, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple tree, and lettuce.

55 (new). The polynucleotide according to claim 1, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the amino acid corresponding to position 426 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a phenylalanine amino acid and the amino acid corresponding to position 177 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

56 (new). The polynucleotide according to claim 55, wherein said amino acid corresponding to position 177 is replaced with a proline.

57 (new). The polynucleotide according to claim 55, wherein said amino acid corresponding to position 177 is replaced with a valine.

58 (new). The polynucleotide according to claim 1, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the amino acid corresponding to position 324 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a lysine amino acid, and the amino acid corresponding to position 359 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a valine amino acid, and the amino acid corresponding to position 396 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

59 (new). The polynucleotide according to claim 58, wherein said amino acid corresponding to position 396 is replaced with a valine.

60 (new). A method for increasing resistance of a plant to heat stress conditions, said method comprising incorporating the polynucleotide of claim 1 into the genome of said plant and expressing the protein encoded by said polynucleotide molecule.

61 (new). The method according to claim 60, wherein said plant is a monocotyledonous plant.

62 (new). The method according to claim 61, wherein said monocotyledonous plant is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, lily, and millet.

63 (new). The method according to claim 60, wherein said plant is *Zea mays*.

64 (new). The method according to claim 60, wherein said plant is a dicotyledonous plant.

65 (new). The method according to claim 64, wherein said dicotyledonous plant is selected from the group consisting of pea, alfalfa, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple tree, and lettuce.

66 (new). The method according to claim 60, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the amino acid corresponding to position 426 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a phenylalanine amino acid and the amino acid corresponding to position 177 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

67 (new). The method according to claim 66, wherein said amino acid corresponding to position 177 is replaced with a proline.

68 (new). The method according to claim 66, wherein said acid corresponding to position 177 is replaced with a valine.

69 (new). The method according to claim 60, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the amino acid corresponding to position 324 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a lysine amino acid, and the amino acid corresponding to position 359 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a valine amino acid, and the amino acid corresponding to position 396 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

70 (new). The method according to claim 69, wherein said amino acid corresponding to position 396 is replaced with a valine.

71 (new). The method according to claim 60, wherein said mutant polypeptide encoded by said polynucleotide further comprises an amino acid mutation that confers increased seed weight to a plant expressing said polynucleotide.

72 (new). A plant or plant tissue comprising the polynucleotide molecule of claim 1.

73 (new). The plant or plant tissue according to claim 72, wherein said plant or plant tissue is monocotyledonous.

74 (new). The plant or plant tissue according to claim 73, wherein said monocotyledonous plant or plant tissue is selected from the group consisting of rice, wheat, barley, oats, sorghum, maize, lily, and millet.

75 (new). The plant or plant tissue according to claim 72, wherein said plant is *Zea mays* or said plant tissue is from *Zea mays*.

76 (new). The plant or plant tissue according to claim 72, wherein said plant or plant tissue is dicotyledonous.

77 (new). The plant or plant tissue according to claim 76, wherein said dicotyledonous plant or plant tissue is selected from the group consisting of pea, alfalfa, chickpea, chicory, clover, kale, lentil, soybean, tobacco, potato, sweet potato, radish, cabbage, rape, apple tree, and lettuce.

78 (new). The plant tissue according to claim 72, wherein said plant tissue is a seed.

79 (new). The plant or plant tissue according to claim 72, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the amino acid corresponding to position 426 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a phenylalanine amino acid and the amino acid corresponding to position 177 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

80 (new). The plant or plant tissue according to claim 79, wherein said amino acid corresponding to position 177 is replaced with a proline.

81 (new). The plant or plant tissue according to claim 79, wherein said amino acid corresponding to position 177 is replaced with a valine.

82 (new). The plant or plant tissue according to claim 72, wherein said mutant polypeptide encoded by said polynucleotide comprises an amino acid mutation wherein the amino acid corresponding to position 324 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a lysine amino acid, and the amino acid corresponding to

position 359 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by a valine amino acid, and the amino acid corresponding to position 396 of the wild type large subunit of ADP-glucose pyrophosphorylase polypeptide of maize is replaced by an amino acid that confers said increased heat stability on said mutant enzyme.

83 (new). The plant or plant tissue according to claim 82, wherein said amino acid corresponding to position 396 is replaced with a valine.

84 (new). The plant or plant tissue according to claim 72, wherein said mutant polypeptide encoded by said polynucleotide further comprises an amino acid mutation that confers increased seed weight to a plant expressing said polynucleotide.

Remarks

Claims 1 and 3-41 are pending in the subject application. Applicants acknowledge that claims 4, 27, and 28 have been withdrawn from further consideration as being drawn to a non-elected invention. By this Amendment, Applicants have amended claim 1, canceled claims 3-41, and added new claims 42-84. Support for the amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 1 and 42-84 are currently before the Examiner. Favorable consideration of the pending claims is respectfully requested.

The subject specification has been objected to on the grounds that it does not comply with 37 CFR §1.821(a)(1) and (a)(2) which requires reference in the specification to a particular sequence identifier (SEQ ID NO:) for a nucleic acid or amino acid sequence. The Examiner indicates that sequence identifiers are missing from the legends of the figures or the Brief Description of Figures 2-5 of the subject specification. By this Amendment, Applicants have amended the subject specification to include reference to SEQ ID NOs. in the Brief Description of Figures 2-5 as suggested by the Examiner. Accordingly, reconsideration and withdrawal of the objection is respectfully requested.

The Examiner has also objected to Applicants' claim of priority to U.S. Provisional Application No. 60/085,460, filed May 14, 1998, and U.S. Provisional Application No. 60/031,045, filed November 18, 1996, on the grounds that the '460 and '045 applications were filed more than one year before the filing date of the subject application. As requested by the Examiner, Applicants have amended the "Cross-Reference to Related Applications" section of the specification to clarify that related U.S. Application No. 09/312,433 claims the benefit of provisional Application No. 60/085,460 and related U.S. Application No. 08/972,545 claims the benefit of provisional Application No. 60/031,045. Accordingly, reconsideration and withdrawal of the objection is respectfully requested.

Claims 11-13, 16, 19, 22, 25, 34-36, 38, and 41 are objected to because of various informalities. Applicants gratefully acknowledge the Examiner's careful review of the claims. In accordance with the Examiner's suggestions, Applicants have amended the claims. Accordingly, reconsideration and withdrawal of the objection is respectfully requested.

Claims 30-36 are objected to under 37 CFR §1.75 as being a substantial duplicate of claims 5-8 and 11-13. Applicants have canceled claims 30-36 and respectfully assert that the new claims submitted herewith are not duplicates. Reconsideration and withdrawal of the objection is respectfully requested.

Claims 1, 3, 5-13, 20-26, and 30-41 are rejected under 35 USC §101 because the claimed invention is directed to non-statutory subject matter. Applicants gratefully acknowledge the Examiner's suggestions of suitable claim language. By this Amendment, Applicants have amended claim 1 to recite that the polynucleotide is "purified." In regard to claim 20 (corresponding to new claim 72), Applicants respectfully assert that the claim does not have to indicate that the polynucleotide is comprised within a construct. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §101 is respectfully requested.

Claims 1, 3, 5-26, and 29-41 are rejected under 35 USC §112, first paragraph, as nonenabled by the subject specification and as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner acknowledges that the specification is enabled and provides written description for certain polynucleotides that encode heat stable AGP large subunits from maize. However, the Examiner asserts that the subject specification does not enable and does not provide written description for polynucleotides encoding heat stable mutants of the small subunit of AGP or for starch biosynthesis enzymes other than AGP or for other plants.

Applicants respectfully assert that the claims are enabled by the subject specification and that there is adequate written description in the subject specification to convey to the ordinarily skilled artisan that they had possession of the claimed invention. Applicants note that claim 1 already recites that the polypeptide encoded by the polynucleotide is a plant ADP-glucose pyrophosphorylase (AGP). By this Amendment, Applicants have amended claim 1 to specify that the mutation is in the large subunit of a plant AGP polypeptide. In addition, Applicants respectfully assert that the subject specification does enable the claimed invention for plants other than maize. The sequences of large subunit AGP for numerous plant species other than maize were well known in the art prior to the earliest effective filing date of the subject application. As the Examiner is undoubtedly aware, there

is no requirement that a specification teach that which is well known in the art. *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81 (Fed. Cir. 1986) citing *Lindemann Maschinenfabrik v. American Hoist and Derrick*, 221 USPQ 481 (Fed. Cir. 1984), (". . . a patent need not teach, and preferably omits, what is well known in the art."). As shown in the sequence alignment in Figure 2 of the subject application, the amino acids for wheat and barley corresponding to the histidine at position 333 of maize AGP large subunit were known in the art. As can also be seen from Figure 2, there is an extensive amount of homology of amino acid sequence between maize, wheat, and barley. Thus, Applicants respectfully assert there is sufficient written description for the claimed polynucleotides and that a person of ordinary skill in the art could readily prepare and use polynucleotides encoding mutant large subunit AGP of the invention for species other than maize. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §112, first paragraph, is respectfully requested.

Claims 1, 3, 5-26, and 29-41 are rejected under 35 USC §112, second paragraph, as indefinite. Applicants respectfully submit that the claims are definite. However, Applicants have submitted new claims 42-84 and respectfully assert that the claims are not vague and indefinite. In regard to the Examiner's assertion that the recitation of "biologically active fragment" renders claim 1 indefinite, Applicants respectfully assert that the claim refers to a fragment of the mutant polypeptide that has the same or substantially the same biological activity as the full-length mutant polypeptide. Applicants also respectfully assert that the new claims are definite in their recital of an "amino acid corresponding to position -- is replaced with . . .." The ordinarily skilled artisan, having the benefit of the teachings of the subject application, would readily understand the metes and bounds of the claims. Applicants also note that the reference to "the native AGP enzyme subunit" has been deleted from the new claims submitted herewith. Applicants have also amended the claims to delete reference to "prairie grass." Accordingly, reconsideration and withdrawal of the rejection under 35 USC §112, second paragraph, is respectfully requested.

Claim 1 is rejected under 35 USC §102(b) as anticipated by Ballicora *et al.* (1995). The Examiner asserts that the Ballicora *et al.* reference teaches a mutation in the small subunit of potato AGP that results in an enzyme with increased heat stability. Applicants respectfully assert that Ballicora *et al.* reference does not anticipate the claimed invention. However, as noted in r

the rejection under 35 USC §112, Applicants have amended claim 1 to specify that the mutation is in the large subunit of AGP. The Ballicora *et al.* reference does not teach or suggest mutations in the large subunit of AGP that result in increased heat stability. Reconsideration and withdrawal of the rejection under 35 USC §102(b) is respectfully requested.

Claims 1, 3, 5, 30, and 37-39 are rejected under the judicially created doctrine of "obviousness-type" double patenting over claim 1 of U.S. Patent No. 6,069,300. In addition, claims 1, 3, 5, 15-17, 20-23, 26, 30, and 37-39 are rejected under the judicially created doctrine of "obviousness-type" double patenting over claims 1-3, 21-30, and 48-54 of U.S. Patent No. 6,403,863. As noted above, Applicants have canceled claims 3-41. Applicants respectfully assert that the new claims presented herein are not obvious over the cited patents. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

It should be understood that the amendments and new claims presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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DRP/sl

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,809,235 B2

DATED : October 26, 2004

INVENTORS : L. Curtis Hannah, Thomas W. Greene

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1,
Line 46, "25/150°C" should read -- 25/15°C --.

Column 3,
Line 19, "(1 12:1315-1320)" should read -- (112:1315-1320) --.
Line 59, "(Bac, J.M." should read -- (Bae, J.M., --.
Line 65, "[1992] (1992) Plant" should read -- [1992] Plant --.

Column 4,
Line 7, "Sh2 and B:2" should read -- *Sh2* and *Bt2* --.

Column 5,
Line 15, "(SEQ ID NO:2')" should read -- (SEQ ID NO:2) --.
Line 24, "Okita, T.W. Plant" should read -- Okita, T.W. [1996] Plant --.
Line 27, "Preiss, J. J. Biol." should read -- Preiss, J. [1994] J. Biol. --.
Line 48, "amino avid" should read -- amino acid --.

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PATENT NO. 6,809,235
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CERTIFICATE OF CORRECTION

PATENT NO. : 6,809,235 B2

DATED : October 26, 2004

INVENTORS : L. Curtis Hannah, Thomas W. Greene

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 14,
Line 30, " use of Dpn1" should read -- use of DpnI --.

Column 16,
Line 19, "*gig* A$^-$ strain" should read -- *glg* A$^-$ strain --.
Line 26, "*gig* A$^-$ strain" should read -- *glg* A$^-$ strain --.

Column 31,
Line 15, "one seine residue" should read -- one serine residue --.

Column 32,
Line 23, "herein" should read -- wherein --.
Line 43, "one seine residue" should read -- one serine residue --.

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